Cell and Molecular Biology
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Week 05
Cellular Communications
Lecture - 20
Cellular Communication (Part 3)

Hello, everyone. This is Dr. Vishal Trivedi from the Department of Biosciences and Bioengineering, IIT Guwahati. And in this particular module, we are discussing cellular communications. So far, what we have discussed includes the basic mechanism of cellular communications, the different components required for cellular communications, and the relevance of cellular communications in the previous two lectures. We have also discussed the different types of mediators, so we have discussed animal hormones.

So, in the previous lecture, if you recall, we discussed the different types of animal hormones. So we have discussed the peptide-based animal hormones. We had discussed steroid-based animal hormones and then we also discussed a special class of animal hormones. When we were discussing animal hormones, we discussed the different types of hormones, their signaling mechanisms, and their relevance in terms of controlling different types of activity.

Now, in today's lecture, we are going to discuss some more aspects of cell signaling, where we will talk about the special signaling pathways that operate in the different types of pathways. And then we are also going to discuss the plant hormones. And at the end, we are also going to discuss the methods by which you can study cell signaling using different types of techniques. Now, talking about the cellular pathways, there are multiple pathways that are important for cell signaling because cell-to-cell communication is crucial for the coordinated actions of different types of cells. Now one such pathway is called the epithelial mesenchymal transition or EMT.

So EMT is a very, very important pathway that is required for cancer cells to move from their site of origin to different sites within the body, right? And these kinds of events are very important for the cells to gain momentum, and that's how they can actually go from one place to another. So, we'll start the lecture with the discussion of cell signaling in cancer cells; especially, we are going to discuss the EMT. And then, subsequent to that, we are also going to discuss the different types of plant hormones and cell signaling in the plant system. So, as you can see, the cell signaling in the cancer cell, especially what is required for EMT, is important. So the full form of EMT is the epithelial-mesenchymal transition, where the cells that are present in the epithelial type will convert into the

mesenchymal type.

And there's a significant difference between the cells that have epithelial morphology versus mesenchymal morphology because epithelial cells are non-movable. They are actually part of the different types of cellular structures, whereas the mesenchymal origin allows them to move. Right? So they can actually move from one place to another. And that's how you have seen that the cancer cells are going to move from their place of origin to different places; for example, there could be a primary tumor that is formed in the liver or in the lungs. And then from the liver or lung, it is actually going to move to different cellular destinations; for example, it can move to the brain, it can move to the stomach, and it can move to other parts of the body.

So basically, when you talk about the EMT, the cells are going to be of the epithelial type, and then they will actually form a mixed population of epithelial and mesenchymal cells. And then ultimately, they will get converted into the mesenchymal type so that they can gain, you know, the apparatus for movement, right? And that's how they will enter the blood vessels, and then they will actually reach their next destination, where they are going to do the reversal, which means they will be converted from the mesenchymal type to the epithelial type. And that's how they are actually going to have the met transition, and then they are actually going to form the secondary tumor, and that's how their secondary tumor is going to be established in the next organ, right? So this is just a simple example of how the EMT and the MET, which is actually the reversal of the EMT, are going to participate in the establishment of the tumor from its primary site to a secondary site. And this is basically the major reason why cancer cells are very difficult to treat or why cancer cells are so dangerous, right? Because they can go from one part to another part. And that's how they can actually infect the different parts of the body, and according to this, you know, movement, cancer could also be of different grades, right? So EMT is driven by transcriptional programming, right? So this is actually a transcriptional program that will convert an epithelial cell into a mesenchymal cell.

By several signaling pathways, for example, you have the TGF-beta pathway, the BMP pathway, the Wnt and beta-catenin pathway, the Notch pathway, the Hedgehog pathway, and the receptor tyrosine kinase (RTK) pathway. So all of these pathways are being activated when there will be a transcriptional programming. And as a result, the epithelial cells are going to be converted into the mesenchymal cells. So these pathways are responding to the signals.

Right. From the tumor microenvironment, such as growth factors, cytokines, hypoxia, and interaction with the extracellular matrix or ECM. As a result, it is going to initiate the reactions of the EMTs. So we're going to discuss the TGA beta signaling, right, as an

example within the EMT. But apart from TGA beta, you're going to have all these pathways that are actually going to operate simultaneously or in different pathways for different cancer cell types. And that's how they are actually going to be responsible for the EMT pathway.

So in a TGF beta pathway, upon ligand binding, the TGF beta R2 phosphorylates the TGF beta R1 and its GS-rich domain, creating a docking site for SMAD2, SMAD3, and SMAD4. So, this is what is written here. So, you are actually going to have the TGF-beta R2 and TGF-beta R1. And when the TGF beta is going to bind to these receptors, they are actually going to have a cascade of phosphorylation where they are actually first going to generate a docking site for SMAD2 and SMAD3. And as a result of this, the SMAD2 and SMAD3, these transcription factors are then going to be phosphorylated, allowing them to form a complex with SMAD4, right? And then this SMAD complex translocates to the nucleus to regulate the target gene expression.

The complex formation and nuclear imports are mediated by the conserved domain and nuclear localization signals. To regulate the signaling integrity, the TGF beta also activates the inhibitory SMADs like SMAD 6 and SMAD 7, which block further SMAD recruitment. So basically, you have the fine balance of the TGF beta-mediated signaling, which is responsible for the EMT, that is actually going to activate SMAD 2 and 3, and then SMAD 4, and then they will form a complex. This complex will then translocate to the nucleus so that it can delineate further downstream signaling. They also influence the other EMT regulators, such as ZEP and HMGA2, which then regulate SNAI1, SNAI2, and Twist.

TGF-beta signaling also activates SWT2 and reinforces EMT by repressing E-cadherin. So remember that there is a significant difference between the epithelial cells and the mesenchymal cells in terms of their cell surface expression proteins, such as E-cadherin, occludin, and all these proteins that are heavily expressed when they actually have the epithelial phenotype. But when they are converted into mesenchymal cells, or the mesenchymal phenotype, these cells have to be repressed so that they do not bind to each other; they should not attach to each other. Because of that, they will get detached from their primary site, and then they will enter the blood, and that's how they will actually reach the next destination. Apart from that, there are many other kinds of modifications that allow these cells to gain momentum, like their actin morphology; all those fibrils are also going to be developed much better than the epithelial cells.

So this is just a simple example of how the signaling, a very, very complex signaling, regulates a very, very important pathway like the EMT pathway. So far, what we have discussed is about cell signaling, right? And we have discussed cell signaling in the

animal system, where we have discussed hormones, different types of receptors, and so on. And now we are going to start the discussion about how cell signaling works in the plant system. Remember that the plants are also equally responsive as the animals, right? Many people have the perception that plants are not very responsive compared to animals for the simple reason that. When you are actually going to do anything to the animal, they actually show the quick action.

They are actually going to show a movement. They are actually going to respond to your actions and so on, aren't they? But that's something that doesn't happen in the plant because the plants are immobile; they are not going to show you movement, except that if you touch the Mimosa pudica and those kinds of plants, they will actually show movement, and that's how you assume that they are responding. But the plants are also very, very highly responsive, and they also have well-developed cell signaling. The plants are actually having a well-developed self-signaling system for regulating their growth and development. They are also very well developed for responses to environmental stimuli.

Remember that the plants are much more exposed to the environment compared to the animals. Animals have their own houses, their niches. So, because of that, the animals are pretty much protected from environmental stresses. Compared to that, the plants have always been standing in the soil, and they are also going to encounter different types of environmental stress, whether it is drought, high temperature, low temperature, or hypoxia, among other things, right? And then it will also be required to have the different types of molecules so that there will be hormonal communications; they are also required for defense. So remember that the plants are present outside, and they are continuously facing threats from different types of bacteria, viruses, fungi, and so on, right? So they are also having a different type of cell signaling for defense; then the plants are also having coordination among the different organs.

For example, it's also going to have a coordination between the active photosynthesis occurring in the leaves and the plant's conduction of water from the roots, as well as flowering and so on, right? Then it also required cell signaling for the adaptations to the different types of stress. So you have the abiotic stress, you have abiotic stress, and so on. And then we also require the cell signaling for the deregulation of the reproductive processes, which means the formation of the ovum and the formation of the pollen grains. And then when there is fertilization, post-fertilization, there will be cell signaling required for the development of embryos and then the development of embryos into fruits and so on. So, cell signaling is very important in plants because it is required for them to adapt to different types of abiotic and biotic stress.

Now, talking about cell signaling in plants, plants perceive and integrate a wide range of external and internal signals to coordinate growth, development, and stress. So, these are the exogenous signals. These are the endogenous signals. In the exogenous signals, you are actually going to have the light, the mechanical signals, the atmospheric humidity, other plants, soil nutrients, soil water, pathogens, gravity, carbon dioxide, and so on. Within the light, it is going to be responsive to the quality of the light, which means what the wavelength of the light is; then it is going to be responsive to the quantity of the light, which means it is going to respond to the intensity of the light.

Then it's also going to respond to the duration of the light, whether it is low duration versus high duration, which means whether it is going to be eight hours or 16 hours a day. Then it's also going to talk about the direction, isn't it? Whether the light is coming from the east side or the west side. Then, for the mechanical signals, a mechanical signal means some kind of force. So it could be like wind, right, which is actually going to, you know, you have seen that the wind is also swinging the plants, right? Then it could be some herbivores; for example, you have different types of cows, buffaloes, and all sorts of, you know, herbivores like deer. They are also, you know, attacking the plants, disturbing the plants, and causing mechanical disturbances.

And then you're also going to have the atmospheric humidity right; you're going to have the other plant. Remember that the plants are also competing with each other for different types of nutrition. In some cases, the plants are also helping each other by providing different types of nutrients, and so on, so whatever plant is present in the proximity is also going to give them a signal. Then we also have the soil nutrients, so you're going to have different types of nutrients present. You may have the macronutrients or the micronutrients, and so on.

The amount of water present is also going to contribute to the abiotic stress. Then we also have the basic stress, such as different types of pathogens. What is present in the soil? Maybe they're good pathogens, or they're going to be good bacteria. Whether it is the presence of a plant, which is found in the Himalayas, versus the plant that is written about on the seashore, and so on. And then we also have the signal for the gaseous molecules like carbon dioxide, nitrogen, ethylene, and all that, right? And then we have the endogenous signals, so you can have the growth regulators, like different types of hormones such as cytokinin, ethylene, gibberellin, and oxygen.

Additionally, we also have the mechanical endogenous signals, like growth-related tissue compressions and tension. Then we have the different signals like jasmonic acid and salicylic acid; then we have developmental regulators like the mobile RNAs; and then we also have the metabolites like sugars, glutamate, and so on. And all these, whether it is

the exogenous signals or the endogenous signal, are going to regulate growth and development, so let's first start with the first signal, and that is the light signal. So light is a critical environmental cue that influences plant growth, development, and behavior. Plants perceive the light through the specialized photoreceptors that detect the light's HST, wavelength, duration, and directions.

Light signaling pathways transduce the signals to regulate gene expression and coordinate physiological responses. Key developmental processes influenced by the light include seed germination, photomorphogenesis, shade avoidance, and flowering. Now, what are the different types of photoreceptors? So, photoreceptors are the specialized proteins that absorb light and trigger photoresponsiveness in plants. Most constant of a plant protein linked to a light-absorbing chromophore except the UR8, which includes functions without one. They are sensitive to the light wavelength intensity and the photoperiod durations, allowing the plants to adapt to the changing environment.

Photoreceptors regulate the development and protective responses throughout a plant's life. There are different types of photoreceptors, each with varying light sensitivities, such as phytochromes, which respond to red and far-red light. And it is going to absorb in the range of 620 to 800 nanometers. Then we have the cryptochromes, phototropins, and zeolite families, which actually absorb in the range of UV, specifically UVA and blue light. And then you also have the UVR8, which is actually going to absorb UVB light, which is in the range of 280 to 320.

So this is all that these photoreceptors are mostly present on the leaves. And they are actually going to absorb the light of different wavelengths. Like they will be absorbing the UVB, which is between 290 and 315. Then UVA is in the range of 320 to 390. Then you're going to have blue light, which is in the range of 450 to 495.

Then you're going to have a green light, which is in the range of 530 to 570 nanometers. And red and the far-red. And for all these, you are actually going to have different types of photoreceptors. All these photoreceptors are actually going to delineate the downstream signaling, and that's how they are going to control seed germination, seedling de-etiolation, leaf development, flowering, and senescence. So, basically, the primary source of light is the sun, and the sun is actually going to regulate all photoreceptor-mediated responses.

So, this is an example of light signaling: a red light induced seed germination mediated by the photoreceptor phytochrome B. In Arabidopsis thaliana, which is a sample plant, five phytohormones like PhyA, PhyB, and PhyC have been identified. While in some monocots, such as rice and maize, three phytochromes like PhyA, PhyB, and PhyC are

commonly found. While the PhyA is primarily responsible for the forward light absorbance, PhyB is the main regulator of the red light-induced heat germination. Now, what is exactly happening, right? So, seed germination is regulated by a balance between the two key hormones, the abscisic acid-induced dormancy and the gibberellic acid-promoted germination, right? So under the red light, the PhyB is converted from its inactive Pr forms to active Pfr forms, which translocate into the nucleus and cause the degradation of the transcription factor, which is called phytochrome interacting factor 1.

So when there is a red light, which is going to fall, it is actually going to activate both of the photoreceptors, and basically, Phy B is actually going to get converted into phytochrome interacting factor one, and then phytochrome factor one is actually going to inhibit the production of abscisic acid (ABA). At the same time, it is also going to enhance the production of gerbilinic acid through a receptor or GO3OX1, and as a result, it is actually going to promote seed germination. But at the same time, when there is a red light, that red light is going to be absorbed by PhyB, and it is going to form the PFR1, which is going to inhibit the whole reaction, and that is how there is fine control of the light-induced seed germination. So PFI-1 acts as a repressor of C germination by suppressing GA biosynthesis, promoting GA catabolism by activating this, and promoting ABA biosynthesis through the upregulation of ABA-1, NCED6, and NCED9, while inhibiting ABA catabolism by repressing type 3, right? Now, this degradation of PF1 leads to an increased GA level and reduces the ABA level, thereby lifting dormancy and promoting seed germination. Under low light or far-red radiation, 5B is converted back to the inactive PFR form, and the PF1 accumulates, and seed germination is repressed.

Then we have another signaling, which is called stress-linked signaling. So, you have two different types of stress. You have biotic stress. You have an abiotic stress. So abiotic stress includes infections by viruses, bacteria, fungi, nematodes, and attacks by insects or herbivores.

Whereas the abiotic stresses include droughts and flooding, which means low water or high water. Then you have salinity, you have heavy metals, you have UV radiation, you have a nutrient deficiency, you have extreme temperatures, right? And plants constantly face the challenging environmental conditions that can negatively affect their growth, development, and productivity. These stresses are broadly classified as abiotic or biotic stresses. The exposure to such leads to rapid physiological and molecular changes affecting processes like photosynthesis, respiration, flowering, tenescence, and gene expression. To survive and adapt, plants have evolved a complex network that perceives these stresses and triggers the appropriate defenses and tolerance responses.

Then we'll come back to the plant hormones, so it is very, very important that plant hormones are also the good mediators of cell signaling, and that's how they actually regulate the different types of events. Plant hormones, or phytohormones, are organic signaling molecules that regulate plant growth, development, and adaptations. They act at a very low concentration, coordinating the cellular responses to both internal and environmental stress. They are typically synthesized in one tissue and transported to others. They include the classical hormones such as auxin, gibberellin, cytokinin, abscisic acid, or ethylene, and then we also have the newly discovered hormones like the brassinosteroids, jasmonates, salicylates, strigolactone, nitric oxide, polyamines, and the peptide.

So these are the new molecules that are also being found to modulate or downregulate the downstream signaling, and that's how they are actually going to modulate the activities. So, there are five classical plant hormones. You have auxin, cytokinin, gibberellin, abscisic acid, and ethylene. So, let's first talk about the auxins, right? So, auxins are indole-based plant hormones derived primarily from tryptophan. They regulate cell divisions, elongations, and differentiation, influencing the identity and behavior of a plant cell.

Oxygen is primarily synthesized in the shoot apical meristems, leaf primordia, young leaves, and developing seeds, from where it is transported to other tissues to regulate growth and development. It is crucial for processes like phototropism and gravitropism. where uneven oxygen distribution drives directional growth in response to light and gravity. There are natural oxygen compounds. So, you have indole acetic acid, indole butyric acid, 4-chloroindole-3-acetic acid, and then you also have phenyl acetic acid.

So, these are the four compounds that are natural and are found in the system. All these natural compounds actually have an aromatic ring because they are derived from tryptophan; they are derived from indole and contain a carboxymethyl substituent. So this is the structure of the IAA, this is the structure of IBA, this is the structure of PAA, and this is the structure of four-chloroindole acetic acid. So you have indolastic acid, you have IBA, you have 4-chloroindolastic acid, and you have PAA. And this is the molecular formula, right? And this is the structure.

Now, how does the auxin signaling work? So auxin signaling in plants is governed by three core components. TIR1 or AFB, which transport inhibitory protein responses 1 or the auxin signaling F protein, or the auxin receptor. So you're going to have the TIR1. So in response, in the absence of auxin or in the presence of auxin, TIR1 is actually going to co-suppress auxin or endolysic acid transcription. So, ARF, oxygen-responsive factors, the transcription factors, and under low oxygen conditions, the TIR1 or ARB show weak

affinity for oxygen or endolastic acid, allowing oxygen or endolastic acid to bind to the ARF and repress oxygen-responsive gene expression.

So, basically, the oxygen is going to, you know, bind to TRF1, which is actually going to show a weak affinity for the oxygen or the endostatic acid. And that's how there will be oxygen-responsive factors. They are actually going to respond to the oxygen, and that's how they are going to change. The transcriptional activities within the plant are important. As auxin levels increase, the auxin binds to a packet in the RF-AB protein, acting as a molecular glue that enhances its binding to auxin or endolactic acid.

This leads to the polyubic annihilation and degradation of the auxin and release of the RF, which are then free to activate or repress the target genes. This mechanism enables the axon to rapidly and precisely regulate gene expression in response to developmental or environmental factors. Now, what is the function of axons? So, apart from the other types of functions, the axon is very, very important for phototropism and gravitism, right? So, phototropism means the response toward the light, and gravitism is the response toward gravity. So, apical dominance is mediated by the downward movement of auxin from the shoot tip, which suppresses the growth of the auxiliary buds.

So, this is one of the important factors of the auxilin. It promotes cell elongation in the shaded stems or shade avoidance. Then it stimulates flower development, the setting, and ripening of the fruits. A high oxygen level prevents the leaf or fruit from dropping, as declining oxygen permits ethylene to trigger senescence. So this is exactly what happens when there is a sun. If the sun is coming from above, the plant will grow in that direction.

But if the sun is coming from this side, then it is actually going to cause the accumulation of oxygen on one side. Wherever there is an accumulation, it is actually going to allow more movement of this. So it will actually allow the growth of this portion. So there will be a more elongated cell.

It is going to be on this side. And as a result of this, the whole plant is going to move toward the sun side. So, if you have the sun on one side, the sun causes the wind to redistribute away, leading to its accumulation on the shaded side, right? So, basically, it's this side you have a sun, right? Then we'll talk about the next hormone, which is cytokinins. So, cytokinins are a class of adenine-derived plant hormones that primarily stimulate cell division and influence various aspects of growth and development. Synthesized mainly in the roots, they travel upward through the xylem to reach the target tissues such as roots, leaves, and fruits. Cytokines are crucial for regulating cell differentiation, shoot initiation, delay of senescence, and overall plant morphogenesis.

Sites of cytokine biosynthesis and afferents include root tips, developing seeds, young leaves, stems, flowers, silicules, fruits, and shoot meristem. There are natural cytokinin compounds. So you have translethane, cislethane, and you also have isopentanyl adenine. So all of these are actually the adenine derivatives.

This is the structure of the cytokinins. There are different types of cytokinins, aren't there? So you have in nature, the cytokinins are based on an adenine ring with a side chain of isopentenyl attached at the N6 position of the adenine. The presence of a double bond in this side chain leads to the formation of geometric isomers, and zeathene can exist either in a trans-zeathene or a cis-zeathene form. So, basically, this is the adenine structure, right? And then you're also going to have the isopentanyl chain attached to the amino groups present on the adenine, right? Therefore, these are some of the derivatives.

This is a transform. This is a cisform. How does the signaling work in the cytokinase? So cytokinase signaling in plants begins when the cytokinase binds to the membrane-localized histidine kinase receptors AHK2, AHK3, AHK4, and CRE1. So these are the receptors for the cytokinin, which are called AHK2 and AHK3, and these are transmembrane receptors. Upon binding, these receptors autophosphorylate on a conserved histidine residue and transfer the phosphate to a conserved aspartate residue. So, when there is cytokinin binding, it is actually going to initiate the autophosphorylation, right? And autophosphorylation occurs on the aspartate residue. The phosphorylated group is then transferred to the histidine-containing phosphotransfer protein in the cytoplasm.

So it is actually going to, you know, transfer the phosphate group to HP1. And then the HP1 moves into the nucleus and phosphorylates the type B ARR1s, which act as transcriptional regulators of the cytokinin-responsive gene. This system includes a negative feedback loop where the ARR induced by cytokinin signaling acts as a repressor to modulate the responses. So there will be a feedback mechanism where the AHP is also going to initiate the repressive activities, and that's how it is actually going to downregulate the cytokine signaling once the effects are completed or the requirements have already been met. Then we talk about the third hormone, which is called gibberellic acid or gibberellins. So gibberellins are a large family of plant hormones that share a common gibberellin ring and exhibit diverse biological activities.

The most well-known among them is gibberellic acid. Gibberellins regulate various stages of plant growth and development, notably seed germination, seed elongation, leaf expansion, and the flowering site of gibberellin biosynthesis, which occurs in the young tissue of the shoot and developing seeds. These are the key bioactive gibberellins. So, you have GA1, GA3, GA4, GA7. And gibberellins are a large family of 125 structurally

related plant hormones primarily synthesized in the root and shoot apical meristems, young leaves, and the developing seeds. This is the general structure of gibberellic acid; right, so gibberellins are tetracyclic diterpenoid acids that exist in two structural classes.

Those with the 19 or 20 carbon compound, the biologically active forms are usually 19 carbon gibberellins, which feature five-membered lactone rings linking the C4 and C10 due to the loss of C20. The hydroxylation at C3 and C13 significantly enhances the activity of gibberellic acid. Gibberellins act as a primary example, primary examples being dihydroxylated 19-carbon gibberellins. How does the signaling work? So gibberellin acid initiates the signaling by binding to the receptor which is called GID1 or gibberellin insensitive DOF1. So this is the GID1, right? In the presence of GA, the GA-GID complex binds to the del A protein, which is a negative regulator of the GI responses.

This interaction triggers the recruitment of a ubiquitin ligase complex marking the del A comp protein for the degradations. And once the DELLA is degraded, the degradation of the DELLA releases the key transcription factor and other regulatory proteins, such as refoldings, enabling the activation of the GA response signaling. It is actually going to activate the transcription factors, and the transcription factors are then going to activate the GA-responsive genes. In the absence of GA, when GA is not present, the DelA protein remains stable and suppresses transcription. thereby inhibiting growth processes like seed germination, stem elongation, and flowering.

So when the GA is not present, the GID1 is actually going to maintain the DELLA 1, right? And that is actually going to suppress the transcription factors. And that's how it is actually going to prevent any of these processes, which are governed by gibberellic acid, such as seed germination, seed elongation, and flowering. Now, let's come to the next plant hormone, which is the access hormone responsible for dormancy. So, acetic acid is a plant hormone crucial for regulating growth and development.

It controls processes such as leaf senescence, dormancy, and seed germination. It is a plant stress hormone and plays a crucial role in plant responses to environmental stress. Sites of ABA biosynthesis and occurrences include the roots, mature leaves, particularly in response to water stress, and the seeds. The biologically active form of the ABA implant is S-ABA, which has two cysteine 4-trans side chains. The trans-ABA isomer is biologically inactive. ABA is a 15-carbon cis-crotin, and it is unique among the plant hormones for having an asymmetric carbon at position 1, giving rise to the two enantiomers R-ABA and S-ABA.

So this is the structure of the ABA, right? It consists of six small carbon rings with an

attached side chain; right, so this is a side chain, and this is a six-member carbon ring. The naturally occurring and biologically active form of a plant is the S ABA. How does the signaling work? Abscisic acid signaling in the absence of ABA inhibits the phosphatase ABA insensitive and the SnRK2 kinases. And so, in the absence of ABA, the ABA 11, which is a phosphatase, is going to inhibit the activity of this particular kinase, and that is in turn.

So, when the ABA is present, it binds to the pyrabactin resistance1 or PYR1. Pyrabactin resistance1 is like a receptor that then binds to and inhibits AB11, so when the ABA is present, it binds to AB11, which inhibits the activity of AB11. As a result, the kinase SNRK2 will be in an active state, which is how it will actually activate the ABA-responsive factors, leading to changes in transcription. So, this releases the SNRK2 from suppression allowing them to activate the ABA responsive element binding factors or the transcription factors. These ABAs regulate the expression of multiple genes. which is mostly responsible for the dormancy, or they are also responsible for withstanding the different types of stress.

So they are actually going to make the plant withstand different types of stress. So it's actually going to be that ABA is going to generate a lot of protective signals so that, instead of the plant spending its energy on growth and other kinds of activities, it will go into dormancy or withstand the stress by reducing energy production and so on. Then let's move on to the next hormone, which is ethylene. Ethylene is also going to be called a gaseous hormone, right? Because ethylene is actually a gas. Ethylene is a simple gaseous plant hormone that plays a crucial role in coordinating growth, deployment, and stress responses. It is unique among the plant hormones due to its gaseous nature and acts as a signaling molecule in many physiological processes.

The site of ethylene biosynthesis, when occurring, includes the tissue undergoing ripening, such as fruit, roots, and shoots, particularly in response to stress. This is a simple example of the structure of ethylene, which is C_2H_4, right? So this is the structure of C_2H_4, which is ethylene. So ethylene is a simple unsaturated hydrocarbon gas that functions as a naturally occurring plant hormone. And what is the signaling mechanism of ethylene? So, ethylene signaling primarily occurs at the endoplasmic reticulum, where ethylene binds to receptors such as ETR1, ETRS1, ETR2, ERS2, and EIN4, right? So ethylene is a gaseous molecule that, when it enters the cytosol, binds to different types of receptors like ETR1, ETR2, ERF1, ERF2, and EIRN4. And in the absence of ethylene, these receptors activate the kinase called CTR1, which phosphorylates and inactivates EIN2, preventing downstream signaling.

So, when there is no ethylene, it is actually going to activate the RAF-like kinases CTR1,

which are actually going to inactivate the transmembrane proteins EIN1 or EIN2. And that's how it is actually going to inhibit the downstream signaling. But when ethylene is present, right, when C_2H_4 is present, it actually activates EIN2 or downstream signaling. So, that's how it is actually going to activate the ethylene response genes, and that is actually going to regulate plant growth and development, especially fruit ripening.

So fruit ripening is one of the important features of the ethylene hormone-mediated signaling. So, when ethylene is present, it binds to the receptor, leading to CTR1 inactivation. So, this CTR1 is not going to be activated. This allows the C-terminal end of EN2 to be cleaved and translocated to the nucleus, where it activates transcription factors like EN3 and EL1, triggering ethylene-responsive gene expression. So this is all about the different types of mediators. So we discuss the plant mediators in terms of the different types of hormones, abiotic factors, biotic factors, and so on.

We have also discussed the animal system, including the different types of hormones and so on. Now the question is, if there is cell signaling, what are the different weapons or tools available to study the cell signaling events? So, for cell signaling, there are different methods available to study it. These are highly dynamic systems that constantly respond to a wide range of internal and external signals. Remember that cell signaling is a process that occurs continuously. And that is why you require the system that can actually allow you to study these events. Talking about the signal methods, we actually have four different methods: Western blotting, phosphoproteomics, microarray, and the reporter gene.

The applicability and the context in which you can use any of these methods are very, very different, right? In some cases, you can use western blotting. In some cases, you can use phosphoproteomics. In some cases, you can perform a microarray. And in some cases, you can actually use a reporter gene.

We are not going to discuss in detail about these processes, procedures, and so on. But if you like, you can study all these in one of my MOOCs courses, which is called experimental biotechnology. So there's a MOOC course already available where you can actually study experimental biotechnology, where you can study Western blotting, where you can actually study reporter gene assays, and so on. Phosphoproteomics and microarray are probably not present in this particular MOOC course, but you can study them through many excellent and high-quality MOOC courses available on the portal. So, western blotting is a widely used technique to detect a specific protein and analyze its expression or activation during the signaling events.

You have four different steps. You have prepared the sample. Then you are going to

perform the gel filtration. Then you are going to perform the gel electrophoresis. Then you are going to have the membrane transferred. And then you are going to have the immunodetections.

All these processes were discussed in detail in that particular MOOC course. So, in sample preparation, you can do the sample from a single cell. You can do the sample from a tissue and so on, right? Different types of methods are available for how you can prepare the sample from the cell or the tissue. Once you develop the sample, you can then resolve it onto the electrophoresis, like the SDS page, and then you will be able to do the membrane transfer. After that, you will be able to do the immunodetection, where you can use the primary antibody, and you can also use the Secondary antibodies and so on. So there are many technical details available about these events, such as what other different types of SDS-PAGE you are going to use.

What are the different methods for membrane transfer and what are the different types of immunodetections? Because even within the immunodetections, you will have the blocking events, then you will have washing, and then you will have the incubations with the primary, secondary, and so on. So all of this is very, very extensive. So we are not discussing all these events. Then the second method is phosphoproteomics. So, you are going to do phosphoproteomics when you would like to see the response of the global proteins present in a particular cell.

So, remember that the western blotting is what you are going to do for one or two proteins, right? So, that is only when you know which protein is getting affected. But if I want to know, you know, for example, if I add the ABA to the cell, what the proteins are, right? So, if I know about proteins, I can do the western. If I don't know the protein, I want to identify all the proteins, so I should do the phosphoproteomics, especially if I want to know which proteins are going to be phosphorylated. So, phosphoproteomics is concerned with looking at the global picture. Question blocking is going to help see the specific questions or specific problems, right? So, phosphoproteomics is a specialized area of proteomics focused on identifying and analyzing the proteins modified by phosphorylation.

And since phosphorylation often marks when the protein is active or inactive, studying these events helps to understand cellular processes. Unlike the basic protein expression studies, the phosphoproteomics not only reveals which proteins are present but also indicates which signaling pathway may be turned on or turned off. One common method for studying the phosphoproteome is mass spectrometry, which enables the large-scale identification and quantification of phosphorylation events across the proteomes. So, the key steps that are included for the phosphoproteomic are that you are first going to have

the sample preparation and then you are going to have a digestion.

So, cells or tissues are used to extract the proteins, which are then digested into peptides typically using trypsin. Then these are going to be enriched for the phosphopeptides. Since phosphorylated peptides are usually low in abundance, specific enrichment techniques like IMAC or Ti2 affinity chromatography are used to isolate them. Then, once you have done that, you are going to do mass spectrometry to analyze the sizes of those proteins, and then the enriched phosphopeptides are analyzed using high-resolution mass spectrometry to detect their mass and phosphorylation sites. And then you're going to have the data analysis so advanced bioinformatics tools map the phosphorylation sites, quantify the changes, and identify regulated pathways or potential drug targets.

Phosphoproteomics enables the global analysis of the phosphorylation events that drive intracellular signaling. And that is what the main purpose of doing phosphoproteomics is. You are going to see all the proteins present in the cytosol and different organs. Since kinases modify specific amino acids, typically such as serine, threonine, and thioethene, to regulate protein activity, localization, or stability, tracking these modifications provides insight into the pathway. So basically, it is going to give you the key proteins that could be involved, right? And then you can actually do the Western blotting to identify and verify these studies. One method uses the silex or stable isotope labeling by the amino acid in cell culture, a metabolic labeling technique where cells incorporate the non-radioactive isotopically labeled amino acid during growth.

And then, after stimulation, samples are collected over time and analyzed by mass spectrometry to detect the phosphorylation changes across many proteins. This reveals which pathway are activated and help to map the complex signaling network in response to stimuli. So phosphoproteomics is going to give you a global view of phosphoproteins.

OK. And once you know the global phosphoproteins using this data, you will be able to draw the signaling pathway. You can use that in conjunction with the western blotting to reduce the signaling pathways. Then the next technique is microarray. So, this is the technique where we have looked at the global phosphoproteins, correct? Now, if you want to know at the level of genes, then you can actually use these, so this is going to tell you about the global gene expression data. And that is also going to indirectly support your results from the global phosphoproteomic data or from the Western blotting data.

So microarrays are high-throughput laboratory tools to analyze the expression of thousands of genes simultaneously. They consist of a solid surface, usually a glass slide on which thousands of DNA probes are fixed in a grid-like pattern. When labeled nucleic acids from a sample are applied, the complementary sequences bind to the probe,

allowing for the detection and quantification of gene expression across the genome in a single experiment. This technique can help understand gene activities, disease profiling, and cellular responses to various types of stimuli. In a typical microarray experiment, messenger RNA is extracted from the cell and converted into cDNA, which is then fluorescently labeled. This process we have discussed in detail in one of my MOOC courses, which is called molecular biology, right? How can you be able to generate the cDNA from the messenger RNA, right? The labeled cDNA is then applied to a microarray chip containing thousands of DNA probes.

Each probe corresponds to a specific gene. If the cDNA binds to the probe, it indicates that the gene is being expressed in a sample. The chip is then scanned to measure the flow rate intensity, which reflects the level of gene expression. So microarray can help to monitor changes in gene expression across thousands of genes simultaneously, helping to analyze how signaling pathways affect transcriptional activity. When cells are exposed to signals like growth factors, cytokines, or stress stimuli, the downstream transcriptional responses can be understood using the microarray, right? By comparing gene activity before and after a specific signal is introduced, we can see which genes are being turned on or off and figure out which signaling pathways are active, how cells respond to different signals, and what might be going wrong in diseases like cancer. So, the major purpose of doing the microarray is to study the different types of genes that are going to be up-regulated or down-regulated.

You know, studying this data is going to help you understand the signaling pathways being triggered in response to a particular type of stimulus. It could be a stimulus which is, you know, abiotic, or it could be biotic; it could be anything, right? Then the last method is called the reporter gene assay. So, the reporter gene assay is also a confirmation of your microarray data. So once you have the microarray data, you can actually use the reporter gene assays to confirm. So reporter gene assays are mostly used to study the activity of the promoters, right? So remember that all gene expression is being governed by the promoter.

So if you want to know, if you want to study the promoters, the activity of the promoters, because that is only going to tell you which gene is going to be upregulated and downregulated, you can actually study the promoter activity using the reporter gene assays. So, reporter gene assays are widely used to study gene regulation and signaling pathways. And in this method, a reporter gene such as luciferase, EFT, or beta-galactosidase is placed under the control of a specific promoter or regulatory sequence of interest. Which means the promoter that you want to study, right, whether this promoter is a strong promoter or a weak promoter, whether it is going to increase the expression of the gene or downregulate the gene, and so on.

So, when the signaling is activated, it leads to the transcription of the reporter gene, which produces a measurable signal. It could be a light, it could be fluorescence, it could be luminescence, and so on. This can allow for the indirect monitoring of the pathway activity, assessing the reporter strength and the identified responses to stimuli or to the drug. Key steps for one of the commonly used methods for which you can actually be able to construct. So a reporter gene, for example, luciferase or GFP, is cloned downstream of a regulatory event or the promoter.

Then you perform the cell transfection. The construct is introduced into the cell culture using methods such as electroporation, and then stimulation is performed. The cells are then treated with a signaling molecule to activate the pathways. Then you perform the incubations. So cells are allowed to respond, leading to the transcription of the reporter gene if the pathway is active.

So, reporter activity is measured using the appropriate methods. You can actually use luciferase, GFP, and beta-galactosidase. So you can use the luminescence. You can use the GFP like the flow-turn imaging, or you can use the colorimetric assays. Reporter gene assays allow for the easy monitoring of whether a signaling pathway is active or inactive. When the signaling pathway is active, it leads to the expression of the reporter gene, resulting in a measurable signal like light or fluorescence.

The intensity of this signal reflects the strength and activity of a signaling pathway. This approach helps track gene regulation and understand how cells respond to different types of signals. So far, what we have discussed is cell signaling and the methods by which you can study cell signaling. So, if you recall, in this particular module, what have we discussed? We have discussed the relevance and the importance of cell signaling. So we discuss the different types of factors that can potentially trigger cell signaling and how cell signaling regulates the different types of events, whether they are related to growth or to withstanding various types of stress. Whether it is related to delineating the death signals or to the activities related to the activation of the different types of cat spaces, and so on.

And in talking about the plants, we have also discussed how cell signaling regulates the responses to light and different types of biotic and abiotic stresses. and then how the cell signaling mediated by the different types of plant hormones is also regulating the different types of downstream cellular events, such as seed germination, growth, photoperiodism, geotropism, and so on So, with this brief discussion about cell signaling, I would like to conclude my lecture here. In subsequent lectures, we'll discuss some additional aspects related to this particular course. Thank you.